Synthesis and evaluation of two novel rhodamine-based fluorescence probes for specific recognition of Fe^{3+} ion

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ABSTRACT

Two low cytotoxic fluorescence probes Rb1 and Rb2 detecting Fe^{3+} were synthesized and evaluated. Rb1 and Rb2 exhibited an excellent selectivity to Fe^{3+}, which was not disturbed by Ag^{+}, Li^{+}, K^{+}, Na^{+}, NH_{4}^{+}, Fe^{2+}, Pb^{2+}, Ba^{2+}, Cd^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Hg^{2+}, Ca^{2+}, Ce^{3+}, AcO -, Br -, Cl -, HPO_{4}^{2-}, HS0_{3} -, I -, NO_{3} -, S_{2}O_{3}^{2-}, SO_{3}^{2-} and SO_{4}^{2-} ions. The detection limits were 1.87 \times 10^{-7} M for Rb1 and 5.60 \times 10^{-7} M for Rb2, respectively. 1:1 stoichiometry and 1:2 stoichiometry were the most likely recognition mode of Rb1 or Rb2 towards Fe^{3+}, and the corresponding OFF–ON fluorescence mechanisms of Rb1 and Rb2 were proposed.

Introduction

Iron plays a key role in many biochemical processes involving oxygen transport, exotic metabolism mediated by P450 (oxidoreduction), gene expression and signal transduction etc. The overload of iron (especially Fe^{3+}) in the body would cause many severe diseases including hepatitis, cancers, dysfunction of pancreas, livers and heart. While the deficiency of Fe^{3+} might induce anemia, fatigue and decreased immunity. It is worthwhile to note that ferroptosis was recently deemed to be a new strategy for cancer therapy. Also, Fe^{3+} is an important pollutant source of the environment. Therefore, the effective monitoring and detecting iron ions is very significant. Atomic absorption spectroscopy, spectrophotometry, and voltammetry inductively coupled plasma mass spectroscopy were familiar identification method of Fe^{3+}, but limiting their application due to high cost, time-consuming and complex samples. Thus, the fluorescence probe technique for detecting Fe^{3+} ions, as a highly efficient, rapid and convenient technique, has emerged and drawn tremendous attention from academia and industrial circles.

Up to date, various fluorescence probes for selective sensing of Fe^{3+} have been developed, such as fluorescein, quinazolinone, pyrene, rhodamine, coumarin, naphthalimide, boron dipyrromethene difluoride (BODIPY) etc. Among these probes, the fluorescence probes based on rhodamine were the most ideal chemosensor for the detection of iron ions because of their large extinction coefficient, long wavelength absorption and emission, high fluorescence quantum yield and low toxicity. However, few rhodamine-based Fe^{3+} fluorescence probes were reported because the rhodamine probes were easily quenched by the paramagnetism of iron and iron ions were easily interfered by other metal ions such as Cu^{2+} and Hg^{2+} ions. Therefore, the development of a high selective and sensitive sensor for iron ions is still an important challenge. In this work, two novel probes (Rb1 and Rb2) for detecting iron ions were designed and synthesized. The obtained target compounds (Rb1 and Rb2) were correspondingly characterized and evaluated.

Results and discussion

The synthetic routes of probes Rb1 and Rb2 were shown in Schemes 1 and 2. A strong fluorescent rhodamine B, which was used as starting material, first underwent a cyclization process with the ethylenediamine to give a non-fluorescent compound Rh-NH_{2}. Then Rh-NH_{2} reacted with dimethylcarbamoyl chloride to provide the probe Rb1. Similarly, rhodamine B was cyclized by 2-aminoethanol to give a non-fluorescent compound Rh-OH, which was then reacted with dimethylcarbamoyl chloride to provide the probe Rb2. Under the promotion of DIPEA and triphosgene, Rh-OH then...
reacted with 2-morpholinoethan-1-amine to provide probe Rb2. The obtained products were characterized by $^1$H NMR, $^{13}$C NMR spectroscopy, and HRMS spectrometry (Figs. S1–S10).

A strong UV absorption at 564 nm was observed after addition of Fe$^{3+}$ into the solution of Rb1 or Rb2 in EtOH/H$_2$O (8:2, v/v) (50 mM). However, no clear absorption in the range of 500–650 nm was observed upon addition of Ag$^+$, Li$^+$, K$^+$, Na$^+$, NH$_4^+$, Fe$^{2+}$, Ba$^{2+}$, Cd$^{2+}$, Ni$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Mg$^{2+}$, Hg$^{2+}$, Ca$^{2+}$, Cu$^{2+}$ and Ce$^{3+}$ ions into the Rb1 solution, and the addition of Pb$^{2+}$ only showed a weak UV absorption (Fig. 1a). These mean that the fluorescence probe could be ‘turn-on’ by Fe$^{3+}$ ions but was slightly disturbed by Pb$^{2+}$. The addition of these metal ions into the Rb2 solution did not even lead to any absorption in the range of 500–650 nm (Fig. 1b). Fascinatingly, under daylight lamp, the orange red color could be observed for the probe (Rb1 or Rb2) and Fe$^{3+}$ solution with the naked-eye (Fig. 1c), no clear color variation could be observed for the probe (Rb1 or Rb2) in solution (10 $\mu$L) in the solution. As shown in Figs. 2a, 2b, 2c, 3a, 3b, 3c, 4a, 4b, 4c, the fluorescence spectra of Rb1 and Rb2 detecting anions were also analyzed. As shown in Fig. 3, the Rb1 and Rb2 solution showed a strong fluorescence emission at 580 nm ($\lambda_{ex}$ = 530 nm) upon addition of Fe$^{3+}$ ions, and no clear fluorescence emission at 580 nm was observed upon addition of Ag$^+$, Li$^+$, K$^+$, Na$^+$, Fe$^{2+}$, Ba$^{2+}$, Cd$^{2+}$, Ni$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Mg$^{2+}$, Hg$^{2+}$, NH$_4^+$, Ca$^{2+}$, Cu$^{2+}$ and Ce$^{3+}$ ions into the Rb1 solution. The addition of Pb$^{2+}$ showed a weak fluorescence emission at 580 nm ($\lambda_{ex}$ = 530 nm) (Fig. 2a). These mean that the fluorescence probe Rb1 could be ‘turn-on’ by Fe$^{3+}$ ions but was slightly disturbed by Pb$^{2+}$. The addition of these metal ions into the Rb2 solution did not even lead to any fluorescence emission in the range of 500–650 nm (Fig. 2b), only Fe$^{3+}$ gave significant fluorescence response, indicating that probe Rb2 was a ‘turn-on’ fluorescence probe for Fe$^{3+}$. Under the irradiation of ultraviolet lamp (365 nm), the orange red color could be observed for the probe (Rb1 or Rb2) and Fe$^{3+}$ solution with the naked-eye (Fig. 2c), no clear color variation could be observed for the probe (Rb1 or Rb2) and other metal ions solution. It is exciting that the addition of Fe$^{3+}$ could not cause fluorescence enhancement on Rb1 and Rb2 solutions. Therefore, both Rb1 and Rb2 in solution could be recognized by Fe$^{3+}$ and rather than other cations above.

To explore whether the probes (Rb1 and Rb2) could be recognize by anions, AcO$^-$, Br$^-$, Cl$^-$, F$^-$, HCO$_3^-$, HPO$_4^{2-}$, HSO$_3^-$, I$^-$, NO$_3^-$, S$_2$O$_3^{2-}$, SO$_3^{2-}$, SO$_4^{2-}$, the fluorescence spectra of Rb1 and Rb2 detecting anions were also analyzed. As shown in Fig. 4, when Ag$^+$, Li$^+$, K$^+$, Na$^+$, NH$_4^+$, Fe$^{2+}$, Pb$^{2+}$, Ba$^{2+}$, Cd$^{2+}$, Ni$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Mg$^{2+}$, Hg$^{2+}$, Ca$^{2+}$, Cu$^{2+}$, Ce$^{3+}$ ions (100 $\mu$L) were...
added into the test solution with Fe$^{3+}$ ions, no obvious changes in the fluorescence spectra at 580 nm could be observed. Conversely, when the same amount of Fe$^{3+}$ ions were added into the test solution with Ag$^+$, Li$^+$, K$^+$, Fe$^{2+}$, Pb$^{2+}$, Ba$^{2+}$, Cd$^{2+}$, Ce$^{3+}$, Ni$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Fe$^{2+}$, Zn$^{2+}$, Mg$^{2+}$, Hg$^{2+}$, NH$_4^+$, Ca$^{2+}$, Cu$^{2+}$, Na$^+$, the fluorescent intensity of test solution was dramatically enhanced. When AcO$^-$, Br$^-$,
Cl\(^-\), HCO\(_3\), HPO\(_4\)\(^2-\), HSO\(_3\), I\(^-\), NO\(_3\), S\(_2\)O\(_3\)\(^2-\), SO\(_3\)\(^2-\), SO\(_4\)\(^2-\) ions (100 μM) were added into the test solution with Fe\(^{3+}\) ions, no obvious variations in the fluorescence intensity at 580 nm were observed except for HCO\(_3\) (Fig. S11). When HCO\(_3\) was added into the test solution with Fe\(^{3+}\) ions, a double hydrolysis would occur to provide Fe(OH)\(_3\) precipitation, which clearly decreased the fluorescence intensity of the test solution with Fe\(^{3+}\) ions. Therefore, Rb\(_1\) and Rb\(_2\) can be independently used to detect Fe\(^{3+}\) in the solution through judging the color change, absorption and fluorescence, and be not disturbed by the ions listed above except for HCO\(_3\).

To further understand sensitivity of Rb\(_1\) and Rb\(_2\) on Fe\(^{3+}\) ions, the fluorescence titration experiments were carried out. As shown in Fig. 5, with the increasing amount of Fe\(^{3+}\) ions, the fluorescence intensity at 580 nm gradually increased. In the range of 0–60 μM Fe\(^{3+}\), the fluorescence intensity of test solution (Rb\(_1\) and Rb\(_2\)) and Fe\(^{3+}\) concentration showed an excellent linear correlation with \(R_1^2 = 0.987\) (Fig. S12a) and \(R_2^2 = 0.994\) (Fig. S12b). The detection limits of Rb\(_1\) and Rb\(_2\) for Fe\(^{3+}\) were calculated to be 1.87 × 10^{-7} M and 5.24 × 10^{-7} M, respectively, which were low enough to detect a submicromolar concentration of Fe\(^{3+}\). The detection limit is equal to 3S/K, where S is the standard deviation of the blank solution, K is the slope of the curve of the fluorescence intensity versus the sample concentration.\(^{27}\)

To evaluate the cytotoxicity of Rb\(_1\) and Rb\(_2\), cell viability was determined by an MTT method.\(^{28,29}\) where HepG2 cells were incubated with probes Rb\(_1\) and Rb\(_2\) for 24 h. In Fig. 6, Rb\(_1\) and Rb\(_2\) did not show any toxicity to HepG2 cells in 10 μM. When the concentrations of Rb\(_1\) and Rb\(_2\) were 20 μM, Rb\(_1\) slightly showed low toxicity to the cells, and Rb\(_2\) did not still show any toxicity to the cells. Clearly, these two probes could be safely used to detect Fe\(^{3+}\) ions in biological system.

To explore whether the binding of Rb\(_1\) or Rb\(_2\) (50 μM) to Fe\(^{3+}\) (50 μM) was reversible, we chose F\(^-\) to perform the reversible experiments according to the Refs. 30,31 When F\(^-\) ions (12 eq of Fe\(^{3+}\)) were added to the test solution (Rb\(_1\) or Rb\(_2\)) with Fe\(^{3+}\) ions, the fluorescent intensity of the test solution (Rb\(_1\) or Rb\(_2\)) with Fe\(^{3+}\) ions was greatly reduced (Fig. 7). A possible explanation was that Fe\(^{3+}\) could be adequately bound by high concentrations of F\(^-\) to form Na\(_3\)[FeF\(_6\)] complex, inducing that the ring-opening form of Rb\(_1\) or Rb\(_2\) recyclized to give a non-fluorescent spirocyclic Rb\(_1\).
The fluorescence intensity of the test solution was quickly revived when further Fe$^{3+}$ (0.5 eq) was added to the above solutions. Therefore, Rb1 or Rb2 response to Fe$^{3+}$ was reversible, and these two probes could be revived and reused to detect Fe$^{3+}$ ions.

To explore the binding mode of Rb1 or Rb2 towards Fe$^{3+}$, the Job's plot for the fluorescence was outlined with the mole fraction of Fe$^{3+}$ towards Fe$^{3+}$ and probe changing from 0.1 to 0.9, and the total concentration of the Rb1 or Rb2 and Fe$^{3+}$ being maintained at 100 µM. 1:1 stoichiometry was the most likely recognition mode of Rb1 on Fe$^{3+}$ ions (Fig. 8a), and 2:1 stoichiometry was the most likely recognition mode of Rb2 on Fe$^{3+}$ ions (Fig. 8b). The binding process between Rb1 or Rb2 and Fe$^{3+}$ was further proven by HRMS spectroscopy of the complex (Figs. S13–S14). The mass data for Rb1-Fe$^{3+}$ solution showed peaks at 735.23; calcd for C33H41FeN7O9[Rb1 + Fe$^{3+}$ + 2NO3], 735.23096. The mass data for Rb2-Fe$^{3+}$ showed one peak at 1153.61; calcd for C37H57Fe2N10O25[Rb2 $^{3+}$].

**Fig. 7.** Fluorescence spectra of Rb1 (a) and Rb2 (b) (50 µM) in EtOH/H2O (8:2, v/v) in the presence of Fe$^{3+}$ (1 eq) and F$^{-}$ (12 eq).

**Fig. 8.** Job's plot for Rb1-Fe$^{3+}$ complex (a) and Rb2-Fe$^{3+}$ complex (b) (the total concentration of Rb1 and Fe$^{3+}$ or Rb2 and Fe$^{3+}$ was 100 µM).

**Scheme 3.** Proposed recognition mechanism of Rb1, Rb2 towards Fe$^{3+}$.
Eisenstein RS, Dixon SJ, Stockwell BR, Molla HA, Bhowmick R, Katarkar A, Chaudhuri K, Gangopadhyay S, Ali M. Identification of Rb1 or Rb2 towards Fe³⁺ was proposed.

As shown in Scheme 3, Rb1 and Rb2 were remained as non-fluorescent spironolactone form in ETOH/H₂O (8:2, v/v). When Fe³⁺ ions were added into the Rb1 or Rb2 solution, Fe³⁺ bound to the spirocyclic oxygen and the side-chain oxygen or nitrogen of the probes Rb1 or Rb2, causing that the spirocyclic form were opened and emitted strong fluorescence. When a large amount of F⁻ ions were added into the Rb1 or Rb2 solution with Fe³⁺, the ring-opening form of Rb1 or Rb2 recylized to give a non-fluorescent spirocyclic Rb1 or Rb2, which could be ascribed to strong affinity of high concentrations of F⁻ ions on Fe³⁺ ions, resulting in the formation of species [FeF₄]⁷⁻ and the release of free Rb1 or Rb2. Therefore, the identification of Rb1 or Rb2 towards Fe³⁺ was driven by the coordinating effect rather than the catalytic reaction.32,33

Conclusions

In summary, we designed and prepared two novel fluorescence chemosensors Rb1 and Rb2, which exhibited high selectivity and sensitivity towards Fe³⁺. The detection of Rb1 and Rb2 towards Fe³⁺ could be judged by the color change, UV absorption and fluorescence mechanisms of Rb1 or Rb2 towards Fe³⁺, the possible recognition mechanism of Fe³⁺ was bound by Fe³⁺ with a ratio of 1:1, and Rb2 was recognized by Fe³⁺ with a ratio of 1:2, which was confirmed by Job’s plot and HRMS spectroscopy. At last, the corresponding OFF-ON fluorescence mechanisms of Rb1 and Rb2 towards Fe³⁺ ions were proposed.

Acknowledgments

W.J.Y. thanks the support of the National Natural Science Foundation of China (No. 21262004) and Guangxi Key Laboratory of Traditional Chinese Medicine Quality Standards (No. 201602), and W. M. thanks the support of Study Abroad Program for Excellent Ph.D. Students of Guangxi Zhuang Autonomous Region.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2017.05.052.

References
